EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1121

COMPARISON OF CONTROLLED FIELD TEST AEROSOL GENERATION DEVICES TO A LABORATORY DEVICE

Jana Kesavan

RESEARCH AND TECHNOLOGY DIRECTORATE

Andrew McFarland

A. R. MCFARLAND, PHD, PE, INC.
Houston, TX 77069-1127

November 2013

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-11-2013	Final	Oct 2011 – Oct 2012

4. TITLE AND SUBTITLE
Comparison of Controlled Field Test Aerosol Generation Devices to a Laboratory Device

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kesavan, Jana (ECBC); and McFarland, Andrew R. (A. R. McFarland)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRI-A, APG, MD 21010-5424
A. R. McFarland, PhD, PE, Inc., 7119 Mohave Hills, Houston, TX 77069-1127

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1121

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT-LIMIT 200 WORDS:
Biological aerosol detectors are used as early-warning devices to alert personnel to the presence of airborne biological threats. Fielded bioaerosol detectors must be checked periodically with threat-simulant aerosols to confirm their functionality. Two field aerosol generators were available for testing: confidence checker units (CCUs; Flir Systems, Inc.; Elkridge, MD) and puffers, also known as pressurized metered dose inhalers (U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD). In this study, the characteristics of the aerosol output from the field aerosol generators were compared to those of a laboratory aerosol generator, the ink-jet aerosol generator (IJAG). In this study, two similar custom-made proprietary aerosol detectors were used to compare the inter- and intravariability of challenge aerosols generated by eight CCUs, six puffers, and one IJAG. Ten challenges from each aerosol generator were provided to each detector, and the detector responses and alarm statuses were recorded. This report presents the test results and conclusions, which indicate that the CCU outputs had the greatest variability, and the IJAG outputs had the least variability.

15. SUBJECT TERMS:
Confidence checker Metered dose inhaler Ink-jet aerosol generator Aerosol

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Renu B. Rastogi
U	U	U	UU	42	19b. TELEPHONE NUMBER *(include area code)* (410) 436-7545

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EXECUTIVE SUMMARY

Biological aerosol detectors are used as early-warning devices to alert personnel to the presence of airborne biological threats. Detectors monitor the size, concentration, and fluorescence of airborne particles and provide an alarm if an internal data-analysis algorithm identifies a threat. To confirm their functionality, fielded bioaerosol detectors need to be checked periodically with threat-simulant aerosols. Two field aerosol generators were available for testing: confidence checker units (CCUs; Flir Systems, Inc.; Elkridge, MD) and puffers, also known as pressurized metered dose inhalers (U.S. Army Edgewood Chemical Biological Center; Aberdeen Proving Ground, MD). In this study, the output characteristics from field aerosol generators were compared to those of a laboratory aerosol generator, the ink-jet aerosol generator (IJAG). The IJAG can deliver precise particle sizes and concentrations to detectors in a laboratory environment; however, in its current form, the IJAG is not a field-usable instrument. In this study, two similar, custom-made, proprietary aerosol detectors were used to compare the inter- and intravariability of challenge aerosols generated by eight CCUs, six puffers, and one IJAG. Ten challenges from each aerosol generator were provided to each detector, and the responses (in terms of MAX counts) of the detector and the alarm status were recorded. The MAX count is a unitless number that is specific to the proprietary detector system. It takes into account the aerosol size, concentration, and fluorescence. An alarm sounds when the MAX count exceeds a preset threshold value. Challenges were provided only after both detector readings reached zero MAX counts. This allowed for confirmation that the identified challenge differences were due to the challenges alone and were not a result of differing baseline readings.

CCUs are small, battery-operated aerosol generators that are easy to program; however, CCUs produce aerosols with high variability. MAX counts recorded by two detectors ranged from <3.5 (no alarm) to 111.3. The CCUs did not produce an alarm in 5% of the challenges to Detector Unit 1 and 2.5% of the challenges to Detector Unit 2. Analyses indicated that the two detectors measured significantly different outputs from the CCUs ($p = 0.0007$), where the statistical null hypothesis of equality of outputs was rejected if $p < 0.05$. Different CCUs also produced significantly different outputs as measured by the detector units ($p = 5.1 \times 10^{-8}$ for Detector Unit 1; $p = 1.9 \times 10^{-9}$ for Detector Unit 2). Measurements from Detector Unit 1 indicated that outputs from the same CCUs were not statistically different ($p = 0.105$); however, measurements from Detector Unit 2 indicated that outputs from the same CCUs were significantly different ($p = 0.048$).

The second field aerosol generator we tested was a puffer. Puffers are small, field-portable units that produce a fairly consistent aerosol output. Challenges produced by the puffers consisted of 2–7 puffs to the detector, and the challenge process was stopped when an alarm state was achieved. The detector units recorded MAX counts that ranged from 4.2 to 18.4. Analysis indicated that both detector units measured significantly different outputs from the puffers ($p = 0.01$). Different puffers also produced significantly different outputs as measured by the detector units ($p = 5.55 \times 10^{-5}$ for Detector Unit 1; $p = 1.72 \times 10^{-5}$ for Detector Unit 2). Outputs from the same puffers were also significantly different ($p = 0.0018$ for Detector Unit 1; $p = 0.00025$ for Detector Unit 2).

The laboratory aerosol generator that we tested in this study was the IJAG, which is a large laboratory system that requires a trained operator. The MAX count readings recorded by the detector units ranged from <3.5 (no alarm) to 14, with 5% of the challenges not providing an alarm. The detector units recorded significantly different MAX count readings for the IJAG challenges ($p = 0.03$); however, repeated challenges on each detector unit did not produce significantly different MAX count readings ($p = 0.066$). This demonstrated that the IJAG challenges were very similar, but that each detector responded differently.

PREFACE

The work described in this report was started in October 2011 and completed in October 2012. The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

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COMPARISON OF CONTROLLED FIELD TEST AEROSOL GENERATION DEVICES TO A LABORATORY DEVICE

1. INTRODUCTION

Intentional and accidental releases of harmful bioaerosols pose a threat to U.S. soldier and civilian populations. Aerosol detectors identify the presence of threat agents in the air and warn the appropriate personnel; therefore, these systems are fielded in high-priority target areas. These fielded detectors must be checked periodically (with simulant bioaerosols) to establish the functionality in the field. Laboratory testing can be performed (with precise aerosol concentrations) to determine the sensitivity and the amount of degradation of the fielded detectors. Many bioaerosol generators are available for laboratory use, but for field use, it is essential to have portable, self-contained generators that require no electric power. These field aerosol generators must be safe for personnel and the environment, and they must be easy for personnel to use with minimum training. They also must produce small but consistent aerosol doses to determine reduced sensitivity of the detector.

Two field aerosol generators were available for this study: confidence checker units (CCUs; Flir Systems, Inc.; Elkridge, MD) and puffers, also known as pressurized dose inhalers (pMDIs; U.S. Army Edgewood Chemical Biological Center [ECBC]; Aberdeen Proving Ground, MD). In this study, the aerosol characteristics of these field aerosol generators were compared with those from a laboratory aerosol generator, the ink-jet aerosol generator (IJAG; ECBC). The IJAG can deliver precise aerosol challenges to detectors in the laboratory; however, in its current form, the IJAG is not a field-usable instrument. In this study, aerosols from the three types of generators were sampled with two similar custom-made, proprietary aerosol detectors (General Dynamics; Falls Church, VA) to evaluate the aerosols and the detector units.

2. MATERIALS AND METHODS

The two detectors (Units 1 and 2) provide an output that is called the maximum count number (MAX count), which is a unitless value that is based on particle fluorescence, concentration, and size. To prevent subversion of the system, the algorithm that determines the MAX count number is not publicly available; therefore, it is not discussed here. An alarm is activated when the MAX count number exceeds a user-defined threshold level. Challenges were provided only after both detector readings reached zero MAX counts. This allowed for confirmation that the identified challenge differences were due to the challenges alone and were not a result of differing baseline readings.

2.1 CCUs

As shown in Figure 1, a CCU is composed of three principal components: (1) a shroud that is placed over the inlet of an aerosol detector, (2) an atomizer, and (3) aqueous-based challenge material. The inlet shroud is a lightweight, injection-molded plastic that is designed for a specific detector system. It replaces the rain cap of the detector, and it allows airflow through the region between its exhaust tube and the detector inlet tube.

The atomizer (Aerogen; Dangan, Galway, Ireland) produces particles with an average mass median aerodynamic diameter (AD) of 3.6 µm (Aerogen, 2012). This aerosol generator is a low-cost, compact, and low-power device that is used in medical aerosol-delivery applications. The aerosol generator contains a wafer-thin disc with precision-formed tapered holes that are surrounded by a piezoelectric transducer. When an oscillating electrical signal is applied to the transducer, the disc vibrates at a specific frequency and draws the fluid into the microscopic holes. As the disc deflects, the liquid is ejected; it then forms a microscopic liquid stream that breaks into micrometer-sized droplets. The aerosol output is controlled by the pulse number and duration.

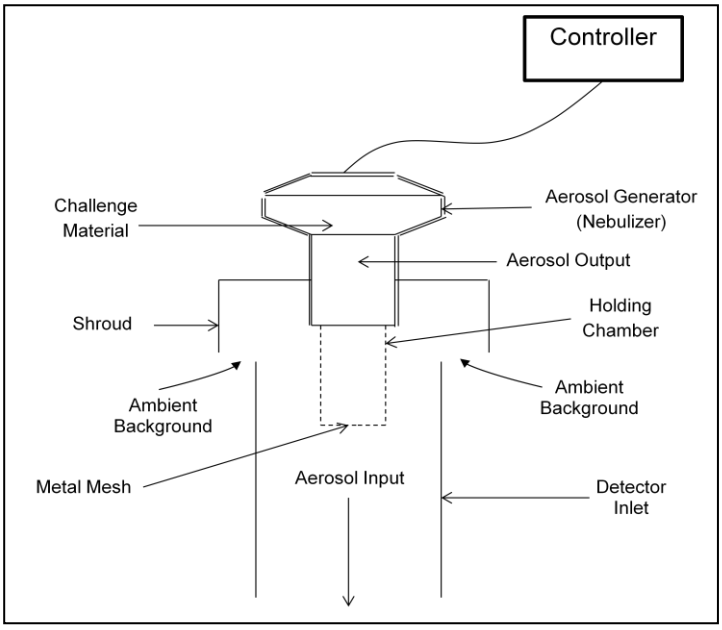

Figure 1. CCU mounted on the inlet tube of a bioaerosol detector.

Many materials, including polystyrene latex (PSL) microspheres, can be aerosolized using CCUs; however, Flir Systems (the vendor) provided two solutions for use in this test. One vial contained 500 mg/mL urea, and the other vial contained a mixture of 1.28 mg/mL riboflavin and 1 mg/mL tryptophan. Ultimately, the vial contents were combined and used in the nebulizer to produce aerosols. The vials of solution were stored in a refrigerator. Before use, both vials were removed from the refrigerator and placed in warm water until each had warmed to room temperature. This step was especially important for urea, which crystallizes when cold. An equal amount of each solution was pipetted into a 1.5 mL centrifuge tube that was briefly vortexed to ensure proper mixing. This solution (0.5–1 mL) was loaded in the nebulizer to produce the aerosol output.

The CCU can be programmed to generate aerosols for different lengths of time and at different rates. In this study, the CCUs were programmed to provide a 100 ms input pulse to the nebulizer to generate the aerosol. A holding chamber (a cylindrical tube with a 100 mesh screen on the bottom) was attached to the nebulizer outlet to remove most of the generated particles. To minimize variability, the same holding chamber was used with all of the CCUs.

2.2 Puffers

The medical version of the pMDI is an inexpensive, self-contained, easy-to-operate, lightweight, portable device that is used by patients for administering inhalable medications. Previous tests have shown that pMDIs provide consistent doses (amounts of pharmaceutical delivered per actuation of a metering valve) of liquid medications during the life of the pMDI (Rubin and Fink, 2005). The pMDI contents are protected against external pathogenic and nonpathogenic contamination by high internal pressure (on the order of several atmospheres). This pMDI technology has been employed for generating non-pharmaceutical aerosols, especially for field testing of biological aerosol sampler detector and identifier systems. The pMDI devices loaded with substances other than medications are referred to as puffers to differentiate them from human-use pMDIs. Vervaet and Byron (2000) filled pMDI canisters with suspensions of 1, 3, 5, and 8 µm fluorescent PSL microspheres and showed that suspensions were stable for 6 months at room-temperature conditions. Carrera et al. (2005) prepared devices with *Bacillus atrophaeus* (also known as *Bacillus subtilis* var. *niger* and *Bacillus globigii* [BG]) spores, which are used as a simulants for pathogenic *Bacillus anthracis* spores because of their similar size and physical properties.

Each puffer (Figure 2) has a nominal capacity of 10 mL. For these tests, the aluminum canister (5.06 g) was filled with 11.72 g (9.55 mL) of HFA-134a (1,1,1,2-tetrafluoroethane) propellant and 0.5 mL of the challenge material to be aerosolized. Puffer preparation and filling were performed in accordance with the techniques of Byron (1994) using a Pamasol P2005 small-scale production unit (Pamasol Willi Mäder AG, Pfäffikon, Switzerland) at ECBC.

Each puffer was fitted with a metering valve and had a nominal release of 50 µL per actuation (model BK357; Bespak Pharmaceutical; Cary, NC). This optimally provided about 200 actuations for the approximately 10 mL puffers. The aerosol is released by movement of the stem into the metering valve. Puffers used in these tests were filled with 0.5 mL of mixture containing 3 µm PSL microspheres and BG DNA, which produced approximately 3 µm microspheres coated with BG DNA. Additional information about puffers, including the amount of output provided, is provided in a separate technical report (Kesavan et al., 2012).

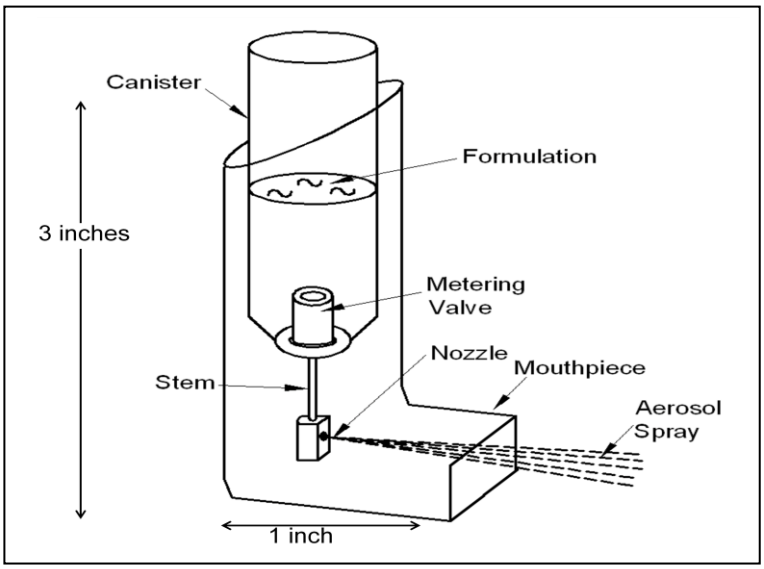

Figure 2. Schematic of a puffer.

2.3 Puffer Mixture Preparation

The liquid solution used to fill the puffers was made by washing nonfluorescent PSL microspheres (Polysciences, Inc.; Warrington, PA) in ethanol to eliminate the aqueous component of the as-delivered PSL hydrosol. This was achieved by adding ethanol to the as-received hydrosol and then centrifuging for 5 min to settle the PSL microspheres. The supernatant was discarded, and the settled microspheres were resuspended in ethanol. This procedure was repeated three times. Ethanol was added for a final PSL concentration of 4×10^8 microspheres/mL. BG DNA was prepared by starting with a γ-killed BG (lot 040; Dugway Proving Ground; Dugway, UT). Initially in a hydrosol state, γ-killed BG was resuspended in ethanol by following the same procedure as that used for PSL. The DNA was then removed from the cells using a bead-beating procedure that consists of adding glass beads to the suspension, vortexing for 1 min, and cooling on ice for 1 min. The vortexing and cooling were conducted five times to completely open the cells and free the DNA. The final mixture was centrifuged for 5 min to settle big particles, cell fragments, and glass beads. DNA from a BG suspension, equivalent to a concentration of 4.8×10^9 organisms/mL, was removed and added to the PSL microsphere suspension for filling the puffers.

2.4 IJAG

The IJAG was developed to enable testing of bioaerosol detection instruments with challenge particle concentrations as low as a few particles per liter. Conventional aerosol testing in an instrumented chamber is difficult at such low concentrations because conventional bioaerosol generators have high aerosol production rates. The IJAG challenge particles were generated one at a time and were seeded directly into the intake flow of the detector being tested. Particle generation rates ranged from arbitrarily low values up to 500 s^{-1}. The rate was based on the frequency of electrical pulses applied to the ink-jet cartridge through the LabView control software (National Instruments; Austin, TX). The airflow rate through the IJAG was controlled to eliminate satellite particles and enhance the delivery of the generated particles.

As shown in Figure 3, the IJAG system is comprised of three principal components. The first component, the dispenser, is the heart of the system. The dispenser is a tubular device that contains the ink-jet cartridge (mounted on top), the light-scattering detector (below the cartridge), and the oven. A high-energy particulate air (HEPA)-filtered carrier flow, typically 1 L/min, transports the particles through the dispenser. It takes about 3 s for a particle to travel the length of the dispenser and exit through a 15.8 mm (0.625 in.) diameter outlet tube. The second component, the controller, provides the dispenser with airflows, the electrical power for the oven, and the pulses to fire the cartridge nozzles. It also provides signal processing for the light-scattering detector. The third component, a computer, operates the IJAG through the controller via a counter/timer PCMCIA card. The IJAG program uses feedback from the light-scattering detector to adjust the nozzle firing rate and achieve the desired particle generation rate, even if one (or more) cartridge jet became nonfunctional.

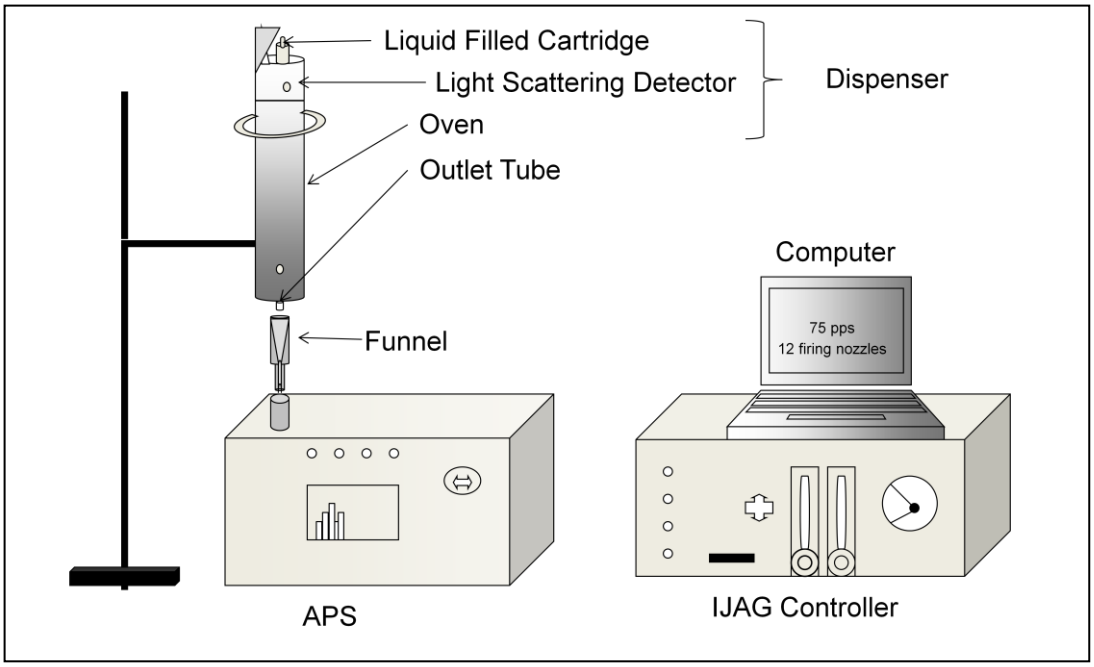

Figure 3. The IJAG system produced particles for measurement by the aerodynamic particle sizer (APS).

The IJAG system includes an HP ThinkJet 12-nozzle printer cartridge (Hewlett-Packard Company; Palo Alto, CA). The cartridge is filled with a dilute aqueous suspension (or solution) of the desired particle formation material at a concentration that would yield the appropriate final residue particle size when dry. This cartridge produces nearly monodispersed primary droplets that are approximately 65 µm in diameter and significant numbers of smaller satellite droplets. Near the top of the oven (Figure 3), a counterflow aperture removes the small satellite droplets that were inevitably produced by the bubble-jet mechanism, leaving only the primary droplets to continue into the oven. Below the counterflow aperture, a light-scattering system detects and counts droplets as they enter the top of the drying oven. The IJAG counts the large original droplets before drying commences, the count is independent of the ultimate residue particle size.

The IJAG is mounted to eject the droplets downward. The primary droplets leaving the cartridge enter a vertical tubular drying oven that is 300 mm (1 ft) high and is heated to approximately 72 °C. As the droplets travel downward through the oven, the nonvolatile contents coagulate into compact spherical solid particles, liquid droplets, or approximately spherical aggregates. Evaporation of the volatile components of the primary droplets result in residue particles of sizes from 1 to 15 μm in diameter, based on solute or suspended particle concentrations of about 0.004 to 1%.

2.5 Size Distribution Measurement of Generated Aerosol Particles

The particle size distributions of the aerosols released by the three generators were measured with an aerodynamic particle sizer (APS; TSI, Inc.; Shoreview, MN) in a HEPA-filtered, clean-air environment. Aerosols generated by the CCUs and puffers were wet; therefore, a custom-made spherical chamber with a volume of 118 L was installed at the APS inlet to dry the particles before they entered the APS. The IJAG included a heated tube that produced dry particles, which were delivered directly to the APS inner nozzle for particle size distribution measurements.

2.6 Inter- and Intravariability of CCU Aerosol Releases

The output inter- and intravariability of aerosol releases from CCU were determined using eight CCUs. For each CCU, the following protocol was followed:

(1) Two initial aerosol releases were generated and discarded;
(2) One release (the "challenge") was delivered to Detector Unit 1;
(3) Two more releases were discarded;
(4) One release was delivered to Detector Unit 2; and
(5) Steps 1–4 were repeated 10 times.

Because each detector unit required 20 min to return to baseline levels, the time between challenges to a given detector unit was at least 20 min. The MAX count and the alarm status of the detector unit for each challenge were recorded and used for later analysis.

2.7 Inter- and Intravariability of Puffer Aerosol Releases

From a batch of 10 puffers, 6 puffers containing PSL microspheres and BG DNA were randomly chosen for this test. The puffers tested were numbers 0, 3, 4, 5, 6, and 7. A flexible aluminum tube with a length of 1.32 m (52 in.) and a diameter of 102 mm (4 in.) was used to contain and direct the aerosol released by a puffer into the aerosol detector. The aluminum tube was shaped such that the horizontal section extended about 1 m (40 in.), and the remaining 0.3 m (12 in.) followed a gentle 90° curve down to the inlet of the aerosol detector. The tube opening was placed completely and securely over the aerosol inlet of the detector to prevent escape of particles. This tube length allowed for the generated particles to dry to final size before they entered the aerosol detector.

The inter- and intravariability of aerosols delivered by puffers was determined in this test. For each puffer, the following protocol was followed:

(1) Two initial aerosol releases were generated and discarded in a biological safety cabinet;
(2) Aerosol releases 2 through 7 were delivered to Detector Unit 1 (the "challenge") until the detector produced an alarm; and
(3) Steps 1 and 2 were repeated 10 times.

Here also, because each detector required 20 min to return to baseline levels, the time between challenges to a given detector was at least 20 min.

2.8 Intervariability of IJAG Aerosol Releases

Before each test, an APS quantified the size and number of particles generated by the IJAG. After this, the IJAG was positioned on top of the aerosol detector such that the bottom of the IJAG outlet was exactly level with the top of the detector inlet, where the rain cap had been removed from the latter. Also, the IJAG outlet was positioned as close as possible to the center of the aerosol detector inlet. The challenge particles were generated at a rate of 100 s^{-1} for 55 s to challenge the aerosol detector. The test protocol consisted of measuring the particles by the APS and then presenting challenges to the aerosol detector units. The sizing and challenges were repeated for a total of 10 trials with each aerosol detector unit. Each detector unit challenge was separated by at least 20 min to allow the detector units to return to baseline conditions. For each challenge, the MAX count and alarm status of the detector unit were recorded and used for analysis. Statistical analyses were conducted to determine inter- and intravariability of the aerosol generators.

2.9 Statistical Analysis

A two-factor analysis of variance was conducted to determine the inter- and intravariability of output produced by the three aerosol generators on each detector unit. A separate *t*-test was conducted to determine the differences between the readings recorded by both detector units.

3. RESULTS

3.1 Particle Size Distributions

Typical particle size distributions of aerosols generated by CCUs, puffers, and the IJAG are shown in Figures 4, 5, and 6, respectively. The average median ADs and geometric standard deviations for the number distributions are presented in Table 1. The number mean diameters (NMDs) for the CCU, puffer, and IJAG aerosols were 2.8, 3.4, and 2.8 µm AD, respectively. The puffer NMD value of 3.4 µm AD was somewhat larger than the aerodynamic equivalent diameter of 3.1 µm AD for the PSL spheres used in the aerosolized suspension. Ostensibly, this is attributable to the DNA coating present on the residual particles subsequent to evaporation of the volatile materials. The geometric standard deviations of the puffer and IJAG

aerosols were 1.05 and 1.07, respectively, which allowed the aerosols to be categorized as nearly monodispersed. In contrast, the geometric standard deviation of the CCU number distribution was 1.57, so that aerosol was categorized as polydispersed. The dispersion differences for the CCU particle sizes as compared with those of the puffer and IJAG are apparent in Figures 4–6.

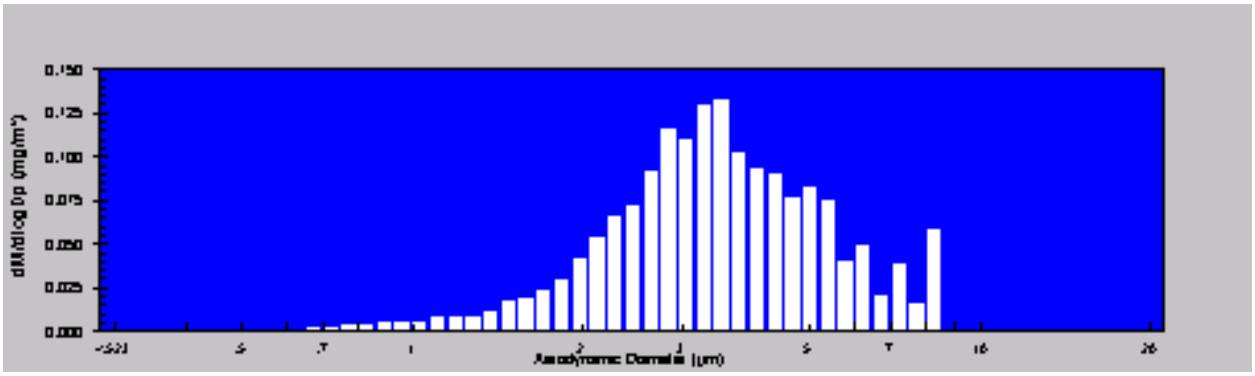

Figure 4. Representative size distribution of particles produced by a CCU.

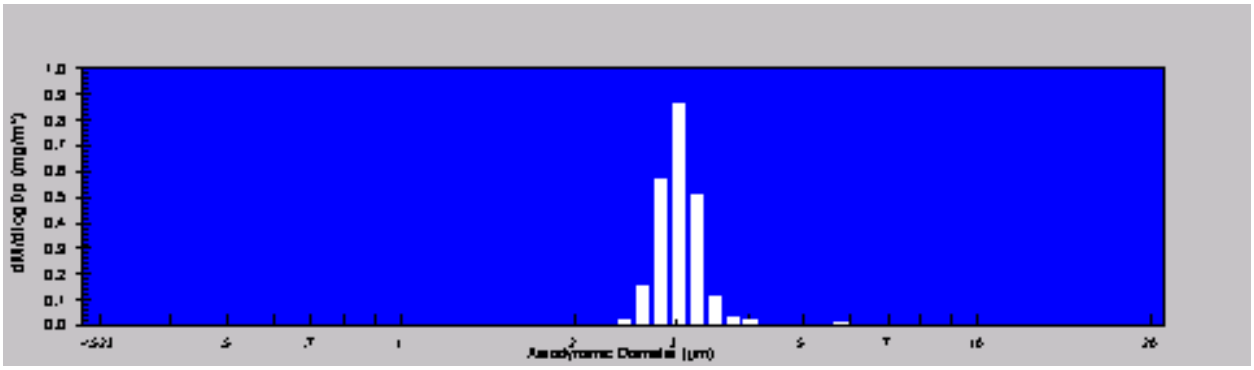

Figure 5. Representative size distribution of particles produced by a puffer.

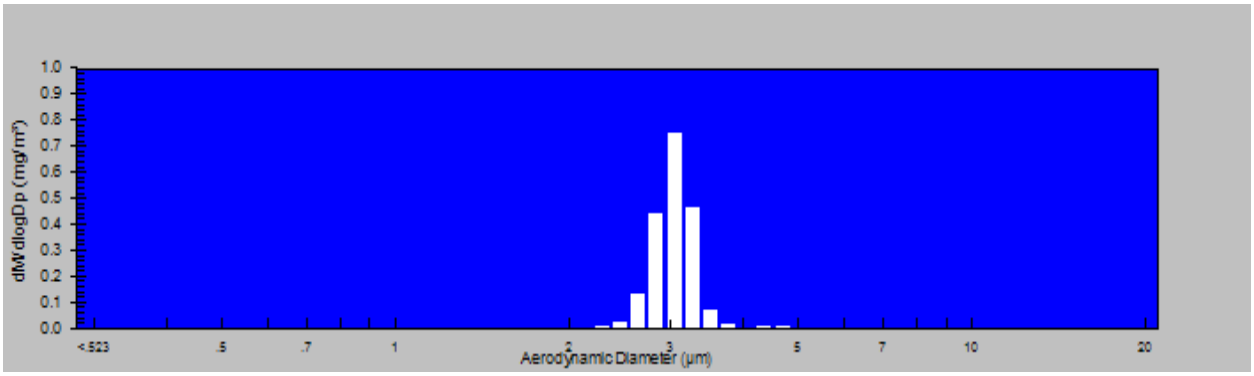

Figure 6. Representative size distribution of particles produced by the IJAG.

Table 1. Size Distribution Parameters of Test Aerosols

Aerosol Generator	Median AD (μm)	Geometric Standard Deviation of Number Distribution
CCU	2.8	1.57
Puffer	3.4	1.05
IJAG	2.8	1.07

3.2 CCU Test Results

The MAX counts recorded by the both detector units for 8 CCUs, which provided 10 challenges each, are shown in Appendix A, Tables A-1 and A-2, and are shown in graphical format in Appendix B, Figures B-1 and B-2. Detector Unit 1 did not produce an alarm for 5% of the challenges, and Detector Unit 2 did not produce an alarm for 2.5% of the challenges. The results indicate that the CCU outputs were highly variable, with MAX counts ranging from <3.5 (no alarm) to 111.3. The minimum, maximum, average, and standard deviation of MAX counts recorded by each detector unit for each CCU are shown in Table 2. The average ±1 standard deviations of the MAX counts are shown in graphical format for each detector unit in Figure 7. The results show that the aerosol delivery was highly variable, with the average MAX counts ranging from 13.1 ± 5.9 to 55.7 ± 26.3 (as shown in Table 2 and Figure 7).

A *t*-test showed that aerosol outputs measured by Detector Units 1 and 2 were statistically different, with $p = 0.0007$. Here, the level of significance was chosen as 5%, meaning the statistical null hypothesis of equality of outputs was rejected if $p < 0.05$. Results of aerosols measured by Detector Unit 1 indicated that different CCUs produced different aerosols ($p = 5.1 \times 10^{-8}$), but the output from the same CCU was not significantly different ($p = 0.11$). Results of aerosols measured by Detector Unit 2 indicated that different CCUs produced statistically different outputs ($p = 1.94 \times 10^{-9}$) and that outputs from the same CCU were slightly statistically different ($p = 0.048$). The CCUs are programmable. Increasing the aerosol-generation time should increase the number of particles, increase the MAX count readings, and eliminate false-negative results.

Table 2. Minimum, Maximum, Average, and Standard Deviation of MAX Count Readings Reported by Detector Units 1 and 2 for Tests with CCUs

CCU No.	Detector Unit 1				Detector Unit 2			
	Minimum	Maximum	Average	SD	Minimum	Maximum	Average	SD
2	3.7	31.4	17.5	9.7	4.4	64.9	24.7	18.9
4	16.0	45.5	28.6	9.6	31.6	61.6	44.5	10.2
7	18.3	39.1	26.7	6.6	7.3	62.9	32.6	15.5
8	13.5	36.1	28.0	6.5	19.7	61.9	38.5	14.8
11	6.0	23.7	13.1	5.9	8.3	27.4	15.2	5.6
18	5.2	29.5	15.8	7.4	5.5	46.4	20.9	14.1
19	4.3	36.3	12.2	8.9	6.4	18.2	12.0	4.2
21	14.5	48.3	30.8	12.4	14.8	111.3	55.7	25.6

SD, standard deviation

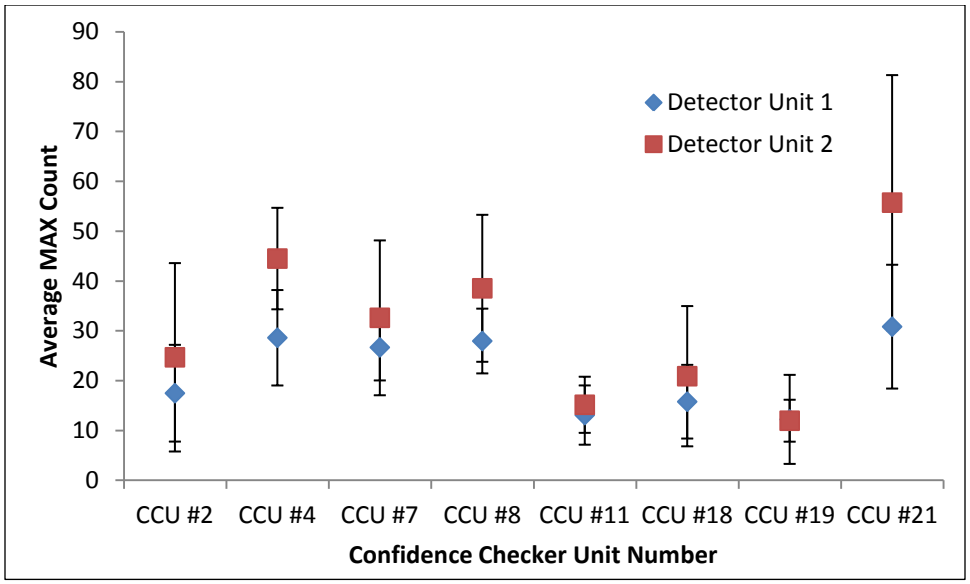

Figure 7. Average MAX count readings for each CCU as measured by Detector Units 1 and 2.

3.3 Puffer Test Results

The MAX count recorded by each detector unit for each puffer challenge is provided in Appendix C, and the data are shown in graphical format in Appendix D. Both detector units produced an alarm for all of the challenges. The MAX counts recorded by both detector units ranged from 4.2 to 18.4 for puffer challenges. The minimum, maximum, average, and standard deviation of MAX count readings recorded by each detector unit for each puffer are shown in Table 3 and Figure 8. The average MAX counts were variable, ranging from 5.1 ± 1.1 to 10.6 ± 3.9.

A *t*-test showed that Detector Units 1 and 2 produced statistically different results, with $p = 0.013$. Results of aerosols measured by Detector Unit 1 indicated that different puffers produced different outputs ($p = 5.55 \times 10^{-5}$) and that outputs from the same puffer were statistically different ($p = 0.002$). Results of aerosols measured by Detector Unit 2 indicated that different puffers produced statistically different outputs ($p = 1.72 \times 10^{-5}$), and the outputs from the same puffer were statistically different ($p = 0.0003$).

The number of puffer actuations needed to produce an alarm by the detector units was variable. For example, 2 puffs from one puffer produced an alarm, whereas 7 puffs from another puffer were required to produce an alarm on the same detector unit. This could have been caused by the puffer orientation within the tube, resulting in varying amounts of aerosol impaction on the walls; however, that factor was not investigated.

Table 3. Minimum, Maximum, Average, and Standard Deviation of MAX Counts Measured by Detector Units 1 and 2 for Puffer Aerosol Releases

Puffer No.	Detector Unit 1				Detector Unit 2			
	Minimum	Maximum	Average	SD	Minimum	Maximum	Average	SD
5	5.6	10.6	8.1	1.5	6.4	11.3	8.4	1.8
0	5.6	8.3	6.5	1.0	4.9	8.5	6.8	1.4
3	4.6	9.1	7.1	1.3	4.2	9.2	5.7	1.6
4	4.8	9.9	7.3	1.5	3.0	6.7	5.1	1.1
7	5.5	18.4	10.6	3.9	4.9	10.7	7.9	2.2
6	5.5	13.6	9.4	2.6	4.6	13.7	8.3	2.9

SD, standard deviation

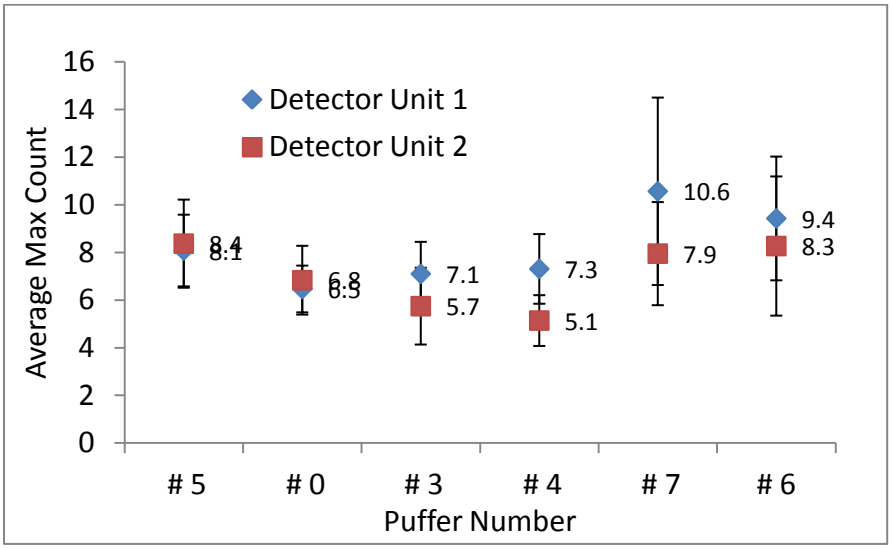

Figure 8. Average MAX count readings reported by Detector Units 1 and 2 for puffer aerosol releases.

3.4 IJAG Test Results

Only 1 IJAG was available for this test; therefore, all 10 challenges were provided by the same IJAG. The MAX count data for each challenge are shown in Appendix E, and the data are shown graphically in Appendix F. Detector Units 1 and 2 (combined) did not produce an alarm 5% of the time. The minimum, maximum, average, and standard deviation values of the MAX count data recorded by each detector unit are shown in Table 4 and Figure 9. The MAX count values were very consistent and ranged from <3.5 (no alarm) to 14.0. Statistical analysis indicated that the aerosols measured by the both detector units were significantly different (p = 0.032). As measured by each detector unit, the IJAG appeared to produce consistent output (p = 0.066).

Table 4. Minimum, Maximum, Average, and Standard Deviation of MAX Counts Reported by Detector Units 1 and 2 for IJAG Aerosol Releases

Detector Unit	Minimum	Maximum	Average	SD
1	3.5	8.4	4.9	1.4
2	3.8	14.0	7.4	3.1

SD, standard deviation

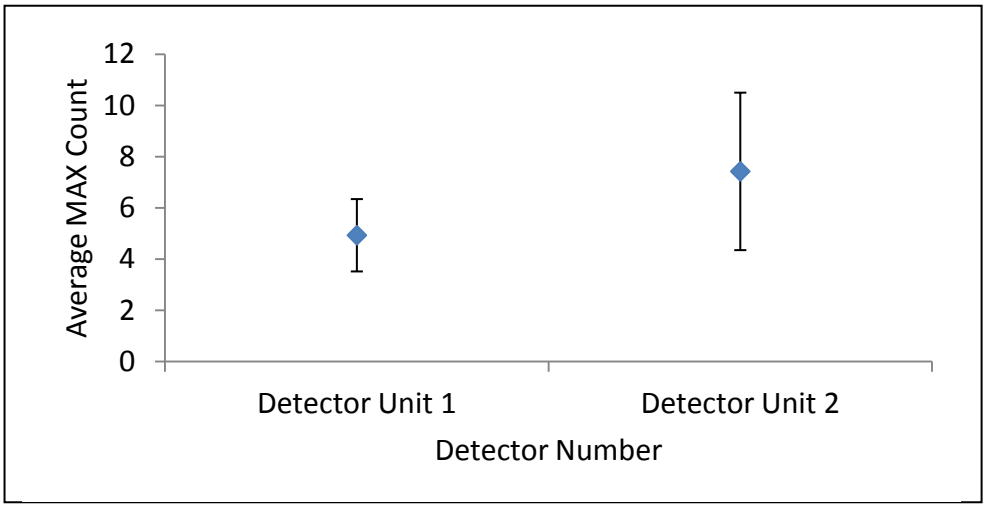

Figure 9. Average MAX count readings reported by Detector Units 1 and 2 for IJAG aerosol releases.

The IJAG-produced aerosol failed to initiate alarms 5% of the time. This seems high, but the IJAG was set to produce very low concentrations of aerosols, which resulted in some challenges that failed to make the detector unit alarm. Increasing the aerosol generation rate by the IJAG would eliminate this problem.

4. SUMMARY AND CONCLUSIONS

The study evaluated three aerosol generators for testing two bioaerosol detector units. Two aerosol generators, CCUs and puffers, are appropriate for field use, and the third unit, the IJAG, is appropriate for laboratory use. All three aerosol generators could be made to produce variable aerosol concentrations by programming the CCUs and the IJAG and by effecting different numbers of releases from the puffers. The conclusions reached from this study, which should help users select the appropriate aerosol delivery system, are listed below.

CCUs are small, battery-operated, portable units that are easy to program but produced aerosols with the highest variability. The MAX counts recorded by the detector units ranged from <3.5 (no alarm) to 111.3. The CCUs did not produce an alarm in 5% of the challenges to Detector Unit 1 and 2.5% of the challenges to Detector Unit 2. Analysis indicated that both detector units measured significantly different outputs from the CCUs (p = 0.0007).

Different CCUs also produced significantly different outputs as measured by the detector units ($p = 5.1 \times 10^{-8}$ for Detector Unit 1, and $p = 1.9 \times 10^{-9}$ for Detector Unit 2). Measurements from Detector Unit 1 indicated that outputs from the same CCUs were not statistically different ($p = 0.105$); however, measurements from Detector Unit 2 suggested that outputs from the same CCUs were significantly different ($p = 0.048$).

Puffers are small, field-portable units that produced fairly consistent aerosols: the detector units recorded MAX counts ranging from 4.2 to 18.4. All of the challenges produced by the puffers, consisting of up to 7 puffs, produced alarms. Analysis indicated that both detector units measured outputs significantly different from those for the puffers ($p = 0.01$). Different puffers also produced significantly different outputs as measured by the detector units ($p = 5.6 \times 10^{-5}$ for Detector Unit 1, and $p = 1.7 \times 10^{-5}$ for Detector Unit 2). Outputs from the same puffers were also significantly different ($p = 0.0018$ for Detector Unit 1 and $p = 0.00025$ for Detector Unit 2).

The IJAG is a laboratory system that requires a trained operator. The MAX count readings recorded by the detector units ranged from <3.5 (no alarm) to 14, with 5% of the challenges not providing an alarm. Both detector units recorded significantly different MAX count readings ($p = 0.03$); however, the repeated challenges did not produce significantly different MAX count readings on each detector unit ($p = 0.066$).

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REFERENCES

Aeroneb Go product page; Aerogen; Dangan, Galway, Ireland. http://www.aerogen.com/aeroneb-go.html (accessed October 2013).

Byron, P. Dosing Reproducibility from Experimental Albuterol Suspension Metered-Dose Inhalers. *Pharm. Res.* **1994**, *11*(4), 580–584.

Carrera, M.; Kesavan, J.; Zandomeni, R.; Sagripanti, J.-L. Method to Determine the Number of Bacterial Spores within Aerosol Particles. *Aerosol. Sci. Technol.* **2005**, *39*, 1–6.

Kesavan, J.; Schepers, D.R.; Bottiger, J.R.; King, M.D.; McFarland, A.R. *Use of Medical Metered Dose Inhalers for Functionality Testing of Bioaerosol Detection and Identification Systems*; ECBC-TR-964; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2012; UNCLASSIFIED Report.

Rubin, B.; Fink, J. Optimizing Aerosol Delivery by Pressurized Metered-Dose Inhalers. *Respir. Care* **2005**, *50*(9), 1191–1200.

Vervaet, C.; Byron, P. Polystyrene Microsphere Spray Standards based on CFC-Free Inhaler Technology. *J. Aerosol Med.* **2000**, *13*(2), 105–115.

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ACRONYMS AND ABBREVIATIONS

AD	aerodynamic diameter
APS	aerodynamic particle sizer
BG	*Bacillus globigii*
CCU	confidence checker unit
ECBC	U.S. Army Edgewood Chemical Biological Center
HEPA	high-energy particulate air
IJAG	ink-jet aerosol generator
MAX count	unitless value based on particle fluorescence, concentration, and size
NMD	number mean diameter
pMDI	pressurized metered dose inhaler
PSL	polystyrene latex

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APPENDIX A
MAX COUNT READINGS FROM DETECTOR UNITS 1 AND 2
FOR ALL CHALLENGES WITH EIGHT CCUs

Table A-1. MAX Count Readings from Detector Unit 1 for Challenges with CCUs

Challenge No.	MAX Count							
	CCU No. 2	CCU No. 4	CCU No. 7	CCU No. 8	CCU No. 11	CCU No. 18	CCU No. 19	CCU No. 21
1	NA	24.1	19.5	23.4	8.5	15.6	5.7	43.7
2	3.7	16.0	27.4	31.3	6.0	10.8	12.4	48.3
3	4.5	21.8	30.9	28.6	7.1	16.9	11.9	22.5
4	10.6	45.5	31.1	34.5	18.8	29.5	8.2	21.9
5	31.4	26.3	39.1	36.1	14.8	17.1	11.7	34.8
6	18.1	33.5	31.9	31.6	23.7	15.3	13.3	33.1
7	21.5	37.1	18.3	23.9	11.1	NA	9.7	14.5
8	27.3	38.7	23.7	13.5	19.8	NA	8.7	21.3
9	23.7	24.1	24.7	28.1	10.1	NA	36.3	47.7
10	16.6	19.0	20.1	28.3	11.1	5.2	4.3	20.6
Minimum	3.7	16.0	18.3	13.5	6.0	5.2	4.3	14.5
Maximum	31.4	45.5	39.1	36.1	23.7	29.5	36.3	48.3
Average	17.5	28.6	26.7	28.0	13.1	15.8	12.2	30.8
SD	9.7	9.6	6.6	6.5	5.9	7.4	8.9	12.4

NA, no alarm
SD, standard deviation

Table A-2. MAX Count Readings from Detector Unit 2 for Challenges with CCUs

Challenge No.	MAX Count							
	CCU No. 2	CCU No. 4	CCU No. 7	CCU No. 8	CCU No. 11	CCU No. 18	CCU No. 19	CCU No. 21
1	4.4	31.6	27.4	47.4	12.6	27.3	10.8	14.8
2	9.0	32.7	37.4	31.2	10.3	14.1	8.3	46.2
3	NA	37.0	49.3	42.3	8.3	37.9	12.8	111.3
4	16.4	49.6	37.7	49.1	14.2	22.3	9.9	48.2
5	19.0	61.6	62.9	61.9	11.1	17.4	12.9	69.9
6	64.9	34.9	33.6	55.7	14.3	46.4	16.7	63.9
7	37.4	50.6	25.9	30.4	20.0	9.2	6.4	61.4
8	20.3	51.3	22.5	21.6	14.2	NA	16.6	29.9
9	37.7	42.7	7.3	19.7	19.2	5.5	18.2	52.5
10	13.1	53.2	22.1	26.1	27.4	7.9	6.9	59.1
Minimum	4.4	31.6	7.3	19.7	8.3	5.5	6.4	14.8
Maximum	64.9	61.6	62.9	61.9	27.4	46.4	18.2	111.3
Average	24.7	44.5	32.6	38.5	15.2	20.9	12.0	55.7
SD	18.9	10.2	15.5	14.8	5.6	14.1	4.2	25.6

NA, no alarm
SD, standard deviation

APPENDIX B
GRAPHICAL REPRESENTATIONS OF DATA OF MAX COUNT READINGS
FROM DETECTOR UNITS 1 AND 2 FOR CHALLENGES WITH EIGHT CCUs

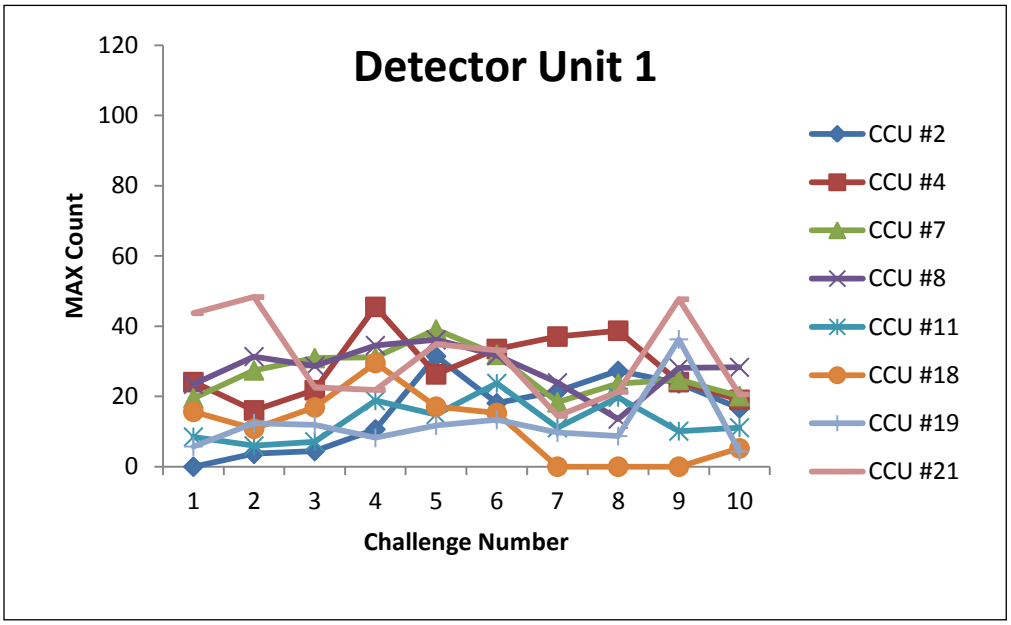

Figure B-1. MAX count readings from Detector Unit 1 for challenges with CCUs.

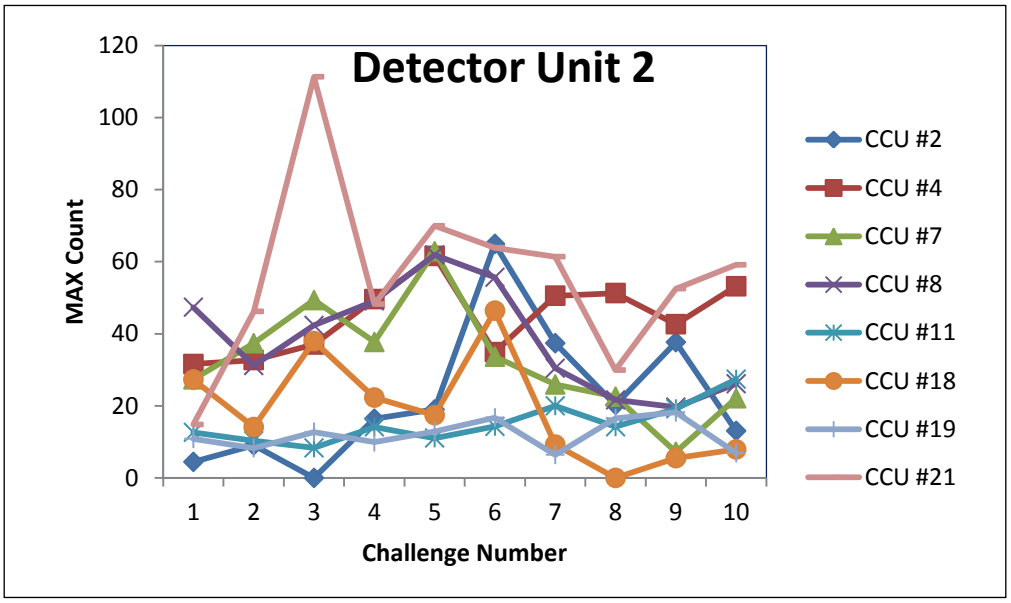

Figure B-2. MAX count readings from Detector Unit 2 for challenges with CCUs.

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APPENDIX C
MAX COUNT READINGS FROM DETECTOR UNITS 1 AND 2
FOR ALL CHALLENGES WITH SIX PUFFERS

Table C-1. MAX Counts Recorded by Detector No. 1 for Challenges by Puffers 5, 0, 3, 4, 7, and 6 in the Order of Testing

Challenge No.	MAX Count					
	Puffer No. 5	Puffer No. 0	Puffer No. 3	Puffer No. 4	Puffer No. 7	Puffer No. 6
1	7.3	5.9	4.2	5.1	4.9	4.6
2	6.8	5.4	4.2	5.0	5.5	5.9
3	7.4	4.9	4.3	5.1	7.8	7.1
4	8.9	8.5	5.4	3.0	6.7	5.7
5	7.7	7.7	4.5	5.2	6.9	7.0
6	9.9	8.3	9.2	5.2	10.3	7.0
7	6.8	5.7	7.1	5.2	6.6	9.8
8	6.4	7.8	6.8	4.2	10.7	11.4
9	11.3	8.4	5.7	6.7	10.0	13.7
10	11.2	5.6	6.0	6.6	10.2	10.4
Minimum	6.4	4.9	4.2	3.0	4.9	4.6
Maximum	11.3	8.5	9.2	6.7	10.7	13.7
Average	8.4	6.8	5.7	5.1	7.9	8.3
SD	1.8	1.4	1.6	1.1	2.2	2.9

Note: The puffer aerosol deliveries (2–7 puffs) were provided until the detector status changed.
SD, standard deviation

Table C-2. MAX Counts Recorded by Detector Unit 2 for Challenges by Puffers 5, 0, 3, 4, 7, and 6 in the Order of Testing

Challenge No.	MAX Count					
	Puffer No. 5	Puffer No. 0	Puffer No. 3	Puffer No. 4	Puffer No. 7	Puffer No. 6
1	8.7	5.6	4.6	4.8	5.5	5.5
2	7.2	5.6	6.0	5.9	8.0	6.4
3	6.7	6.5	5.5	8.6	9.3	8.6
4	5.6	5.6	7.1	7.0	8.6	8.2
5	10.6	8.1	7.5	6.6	6.2	8.8
6	8.0	8.3	7.6	7.7	12.7	8.4
7	9.7	6.9	9.1	6.7	10.3	13.6
8	7.5	6.0	7.7	7.3	14.3	10.7
9	7.5	6.1	7.7	9.9	12.3	12.5
10	9.3	6.1	8.1	8.6	18.4	11.5
Minimum	5.6	5.6	4.6	4.8	5.5	5.5
Maximum	10.6	8.3	9.1	9.9	18.4	13.6
Average	8.1	6.5	7.1	7.3	10.6	9.4
SD	1.5	1.0	1.3	1.5	3.9	2.6

Note: The puffer aerosol deliveries (2–7 puffs) were provided until the detector status changed.
SD, standard deviation

APPENDIX D
GRAPHICAL REPRESENTATIONS OF MAX COUNT READINGS
FROM DETECTOR UNITS 1 AND 2 FOR CHALLENGES WITH SIX PUFFERS

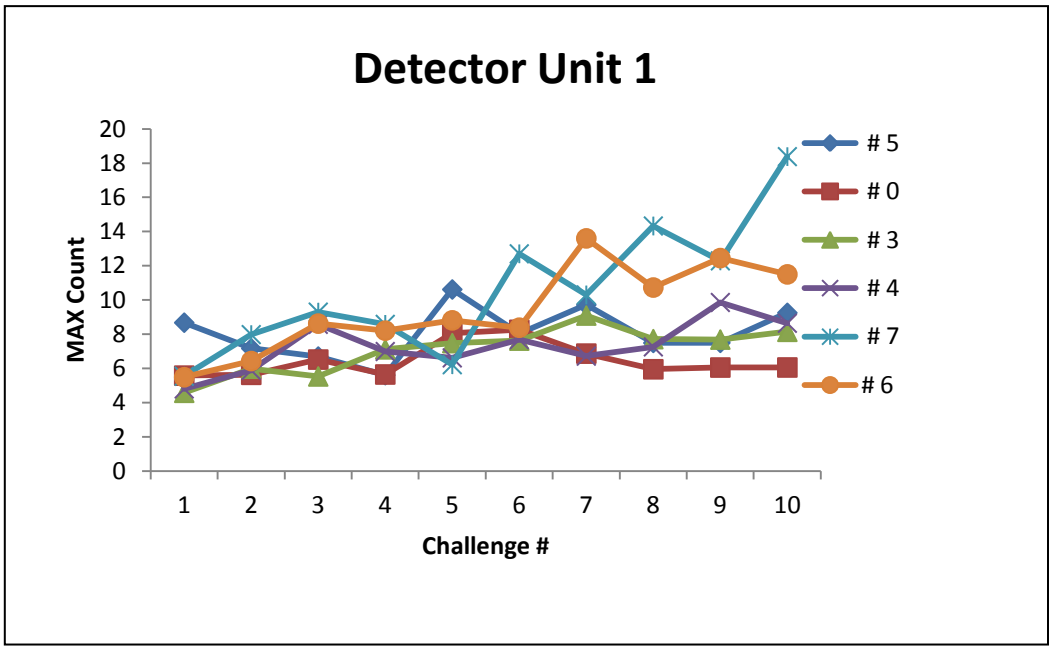

Figure D-1. MAX count readings recorded by Detector Unit 1 for the puffer challenges.

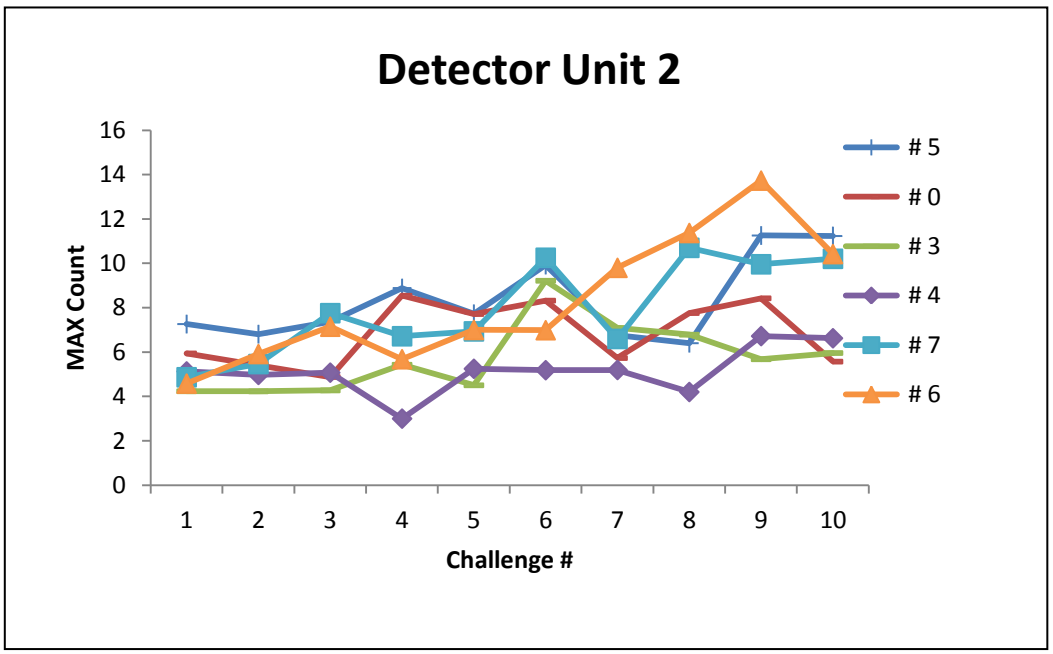

Figure D-2. MAX count readings recorded by Detector Unit 2 for the puffer challenges.

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APPENDIX E
MAX COUNT READINGS REPORTED BY DETECTOR UNITS 1 AND 2 FOR IJAG CHALLENGES

Table E-1. MAX Count Readings Reported by Detector Units 1 and 2 for Particles Produced by the IJAG in Challenges 1–10

Challenge Number	MAX Count	
	Detector Unit No. 1	Detector Unit No. 2
1	4.3	6.1
2	4.8	7.5
3	NA	6.7
4	3.7	5.3
5	4.1	4.3
6	4.3	3.8
7	5.4	14.0
8	8.4	10.9
9	5.4	8.1
10	5.5	7.5
Minimum	3.7	3.8
Maximum	8.4	14.0
Average	4.9	7.4
SD	1.4	3.1

NA, no alarm
SD, standard deviation

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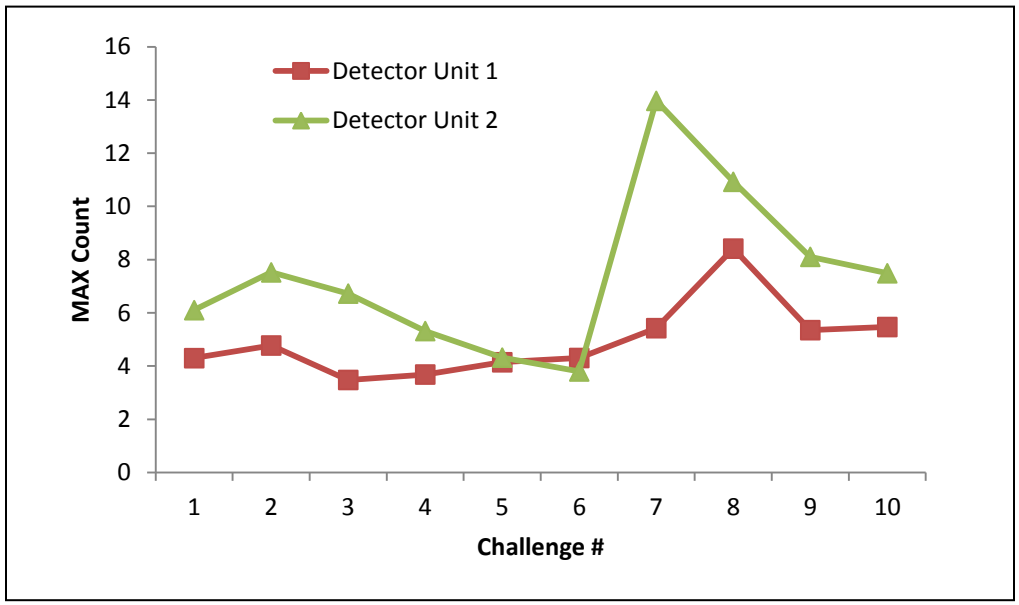
APPENDIX F
GRAPHICAL REPRESENTATION OF DETECTOR UNITS 1 AND 2
FOR IJAG CHALLENGES

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